

## *Cymbidium* × *nomachianum* (Orchidaceae) —A New Natural Hybrid from Japan

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**Abstract** *Cymbidium* × *nomachianum*, a natural hybrid between *Cymbidium aspidistrifolium* Fukuyama and *C. kanran* Makino is newly described from Shikoku District, Japan. All the morphological characters of the new taxon were intermediate between the two species. Patterns of polymorphic sites in nuclear DNA sequences of the new taxon indicated its hybrid origin from *C. aspidistrifolium* and *C. kanran*, and comparisons of plastid DNA sequences among the three taxa suggested the maternal parent status of *C. aspidistrifolium*. Furthermore, artificial hybrid seedlings between *C. aspidistrifolium* and *C. kanran* showed similar vegetative and floral characters to the new taxon.

**Key words:** *Cymbidium*, *Cymbidium* × *nomachianum*, Japan, new natural hybrid, Orchidaceae, taxonomy.

### Introduction

Contrary to the general notion, relatively few natural hybrid species have been recorded in Orchidaceae probably due to its well-developed isolation mechanism by floral characters and sporadic distribution patterns. In Japan, we do not have concrete documentation of hybridization and introgressions in orchid taxa except for cases of putative hybrid swarms in *Calanthe* R. Br. (e.g., Ito and Karasawa, 1969) and *Liparis* Rich. (Tsutsumi and Yukawa, unpublished).

On 6 June 1988, the late Katsuhito Nakanishi found three individuals of unusual *Cymbidium* in a warm-temperate evergreen broad-leaved forest dominated by *Castanopsis sieboldii* (Makino) Hatus. ex T.Yamaz. et Mashiba in Muroto, Kochi Prefecture, Japan, where two *Cymbidium* species, namely, *C. kanran* Makino and *C. aspidistrifolium* Fukuyama grew sympatrically. The plants collected subsequently bloomed and they did not coincide with any hitherto known taxa in *Cymbidium*, whereas characters of the flower seem to be intermediate between *C. kanran* and *C. as-*

*pidistrifolium*. Flowering times of the two species and the new entity overlapped each other in November. We found two other *Cymbidium* species, *C. goeringii* Rchb.f. and *C. nagifolium* Masam., within 10 km of the locality of the new entity. However, their flowering times are March to April and July to August, respectively, and thus there are no chances for them to hybridize with *C. kanran* or *C. aspidistrifolium*. Consequently, we suspected a hybrid origin of the new entity between *C. kanran* and *C. aspidistrifolium* rather than an unrecognized new species. To confirm the status of this taxon, we investigated both morphological and macromolecular characters of it and putative parent species.

### Materials and Methods

Observation and measurement of morphological characters is based on living plants, dried herbarium specimens, and spirit-preserved specimens in Tsukuba Botanical Garden, National Museum of Nature and Science and Kochi Prefectural Makino Botanical Garden.

Table 1. Materials of *Cymbidium* used for DNA sequencing.

Species	Locality	Number of materials
<i>C. kanran</i>	Japan, Shikoku, Kochi Pref., Muroto-shi	2
<i>C. aspidistریفolium</i>	Japan, Shikoku, Kochi Pref., Muroto-shi	2
<i>C. ×nomachianum</i>	Japan, Shikoku, Kochi Pref., Muroto-shi	1

Table 2. Diagnostic characters among *Cymbidium kanran*, *C. ×nomachianum* and *C. aspidistریفolium*.

Character	<i>C. kanran</i>	<i>C. ×nomachianum</i>	<i>C. aspidistریفolium</i>
Length of leaf (cm)	35–73	25–35	14–24
Width of leaf (cm)	1.0–1.8	2.0–2.5	2.5–4.0
Number of leaf	3–5	3–4	2–3
Length of inflorescence (cm)	44–72	10–15	4–10
Number of flowers	5–12	3–4	1–3
Length of dorsal sepal (cm)	4.5–5.1	3.5	2.0–2.5
Width of dorsal sepal (cm)	0.5–0.6	0.5	0.5–0.7
Length of pseudobulb (cm)	2.4–3.0	2.5–3.5	2.6–6.2
Shape of pseudobulb	ovoid	ovoid	clavate
Shape of leaf	linear	lanceolate	broadly oblanceolate
Color of flowers	purplish or green	purplish	pale green

Materials used in the macromolecular analyses are shown in Table 1. Experimental methods followed Yukawa *et al.* (1993, 1996). Sequences were determined by amplifying the ITS (internal transcribed spacer) region of the 18S-26S nuclear ribosomal DNA and a region of the plastid genome encompassing 600 base pairs of the 3' end of *matK* and its flanking *trnK* intron via the polymerase chain reaction (PCR) from a total DNA extract. We used the primers described in Douzery *et al.* (1999) for ITS and in Yukawa *et al.* (1999) for *matK* and *trnK* intron.

### Results and Discussion

Characters discriminating *Cymbidium kanran* and *C. aspidistریفolium*, putative parents of the new entity, were shown in Table 2. All the characters of the new entity were intermediate between *C. kanran* and *C. aspidistریفolium*; therefore, it is highly probable that the new entity is an interspecific hybrid between *C. kanran* and *C. aspidistریفolium*.

DNA sequences of the 3' end of *matK* and *trnK* intron representing the plastid genome were completely identical with *Cymbidium aspidistریفolium* and the new taxon. On the other hand, 15

Table 3. Polymorphic sites found in nrITS sequence alignment of *Cymbidium* examined.

Nucleotide site	Taxon		
	<i>C. aspidistریفolium</i>	<i>C. kanran</i>	<i>C. ×nomachianum</i>
203	G	G	G/T
205	A	T	A/T
253	A	G	G
289	C	C	C/T
550	G	A	A/G
584	C	T	C/T
605	A	G	A/G
635	T	C	C/T
650	G	G	G/T
710	G	A	A/G
718	T	C	C/T
728	T	C	C/T
743	G	A	A/G
782	G	A	A/G

substitutions were detected between *C. kanran* and the others. Regarding the nuclear genome, inconsistent sites in nrITS among the three taxa were shown in Table 3. The results showed that all the inconsistent sites between *C. kanran* and *C. aspidistریفolium* are polymorphic in the new entity except for position 253. More specifically, detected nucleotides from these polymorphic sites of the new taxon were the addition of nu-

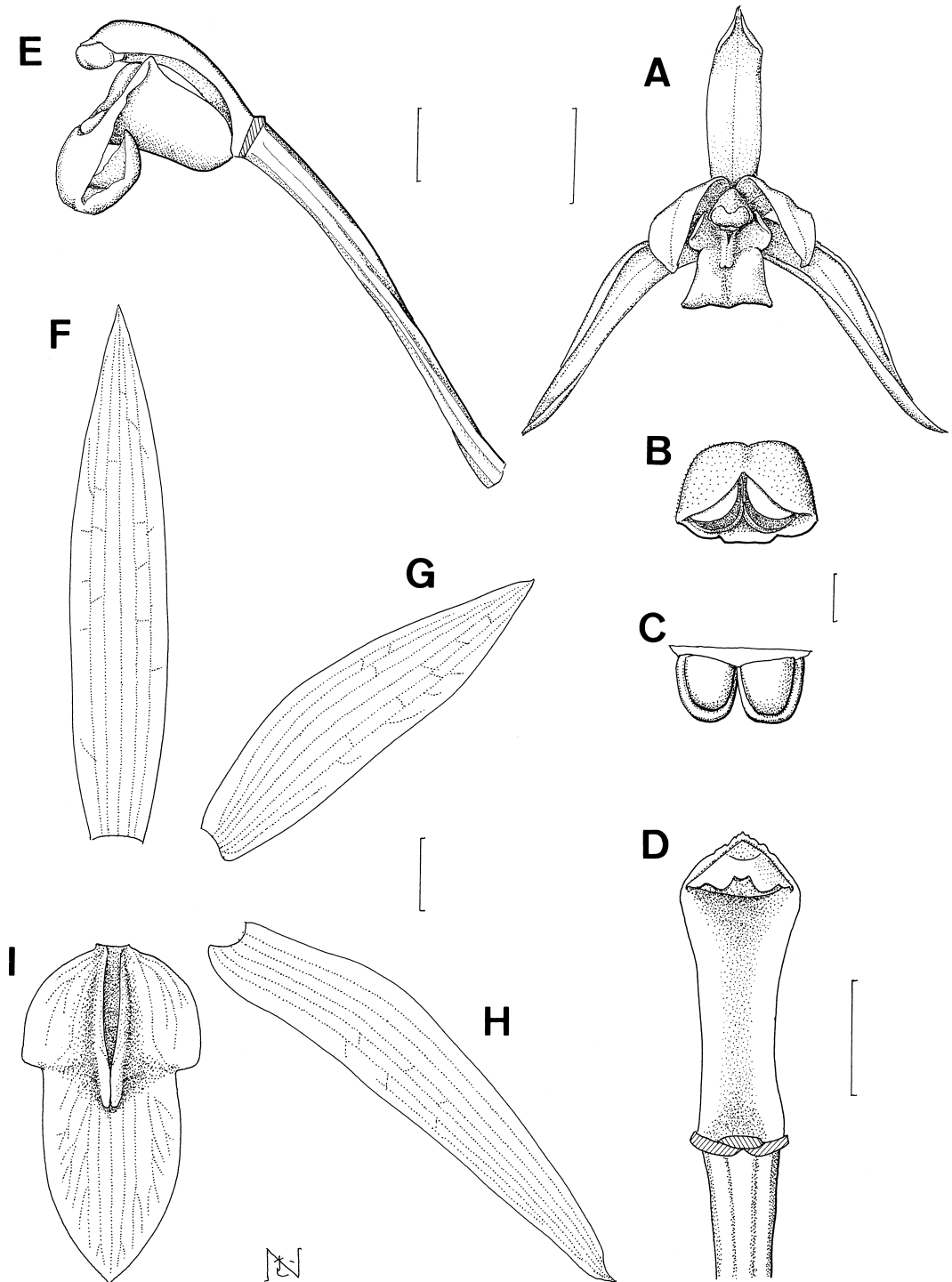


Fig. 1. Flower of *Cymbidium xnomachianum* T. Yukawa & Nb. Tanaka (A–I). A. Flower, front view; B. Anther cap; C. Pollinia; D. Column, from below; E. Labellum and column, side view; F. Dorsal sepal; G. Petal; H. Lateral sepal; I. Labellum. Drawn from holotype (*Hort. Kochi Prefectural Makino Botanical Garden, N. Tanaka 078*) by M. Nakajima. Scale bar=1 cm (A), 5 mm (D–I), or 1 mm (B, C).

cleotides of *C. kanran* and *C. aspidistrifolium*. Positions 203, 289 and 650 of the new entity were polymorphic, whereas these positions were consistent in *C. kanran* and *C. aspidistrifolium*.

Since maternal inheritance of plastid DNA was confirmed in Orchidaceae (Sears, 1980; Corriveau and Coleman, 1988; Cafasso *et al.*, 2005), perfect matching of the new entity and *Cymbidium aspidistrifolium* in plastid DNA sequences suggested that the new entity has a hybrid origin and the maternal parent of it is *C. aspidistrifolium*. On the other hand, patterns of polymorphic sites in nuclear DNA sequences indicated a hybrid origin of the new entity from *C. kanran* and *C. aspidistrifolium*.

No genetic barriers exist between *Cymbidium kanran* and *C. aspidistrifolium* because hybrid seedlings were successfully obtained by artificial hybridization between them (Nomachi, unpublished). He further confirmed that several individuals of the hybrid seedlings showed similar vegetative and floral characters to the new taxon.

All of these results and observations provide convincing evidence for a hybrid origin of this entity between *C. aspidistrifolium* and *C. kanran*. The description of the plant is provided as follows:

***Cymbidium ×nomachianum*** T. Yukawa et Nb. Tanaka, nothosp. nov.

TYPE: JAPAN: Shikoku District, Kochi Pref., Muroto-shi, Kiragawa-cho, Hounoki, alt. ca. 350 m. Flowering in cultivation 26 October 2004, *Hort. Kochi Prefectural Makino Botanical Garden, N. Tanaka 078* (MBK–holotype, TNS–isotype). [Figs. 1, 2]

Hybrida naturalis inter *Cymbidia kanran* Makino et *C. aspidistrifolia* Fukuyama; differt ab anteriore foliis amplioribus et a posteriore florum nervis rubro-purpurascensibus, non viridescensibus, corollis tenuibus, non crassiusculis.

Planta perennis, terrestris, erecta, usque ad 35 cm alta, radicibus flexuosis. Folia 3–4, lanceolata, 25–35 cm longa, 2.0–2.5 cm lata, glabra, apice acuta, basin versus paulatim decurrentia, margine integra. Racemi circa 10–15 cm longi,



Fig. 2. *Cymbidium ×nomachianum* T. Yukawa & Nb. Tanaka. Photograph from holotype (*Hort. Kochi Prefectural Makino Botanical Garden, N. Tanaka 078*).

erecti, glabri, basi vaginis squamiformibus circumdantes, laxe 3–4-flores, bracteis lanceolatis, circa 1.5 cm longis, glaberis, acutis. Flores apertientes, rubro-purpurascens, glabri, 6 cm in diametro. Pedicellis cum ovarioris 2.5–3.0 cm longis. Sepala erecto-patentia, aequilonga, aequilata, lineari-lanceolata, 3.5 cm longa, 0.5 cm lata, glabra, apice acuto-acuminata. Petala ovato-elliptica, 3 cm longa, 0.7 cm lata, non falcata, apice acuta. Labellum carinatum, ovato-oblongum, abrupte curvatum, glabrum, basi rotundatum, apice deltoideo-contractum, supra purpureo-maculatum, 2.5 cm longum. Columuna 1.2 cm longa, recurva. Fructus cylindrica, circa 5 cm longa, 1.5 cm lata.

Japanese name: Nagino-ha-hime-kanran.

Etymology: The specific epithet is named in honour of Atsushi Nomachi, the first to notice the distinctiveness of this natural hybrid.

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